

XLIV. THE AEROBIC AND ANAEROBIC METABOLISM OF THE COMMON COCKROACH (*PERIPLANETA ORIENTALIS*). III.

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INTRODUCTION.

THE common cockroach (*Periplaneta orientalis*) has long been known to live for considerable periods in the complete absence of oxygen. It has further been shown [Davis and Slater, 1926] that during such periods of anaerobiosis the insects go into debt for oxygen in a manner closely resembling that observed in man during severe exercise.

As the cockroaches are perfectly quiescent during the period of anaerobiosis, the debt must be incurred not as the result of special muscular activity, but in maintaining the resting metabolism of the animal as a whole. It is to be expected therefore that the amount of oxygen debt in a given period of anaerobiosis will be equal to the oxygen used at rest in an equal time in air; such a relationship was found to exist.

The investigations of Meyerhof, Warburg and their co-workers on lactic acid formation in isolated tissues suggest the possibility that the energy required for the resting metabolism of the cockroach under anaerobic conditions is supplied by the breakdown of glycogen to lactic acid, with the subsequent removal of lactic acid on the readmission of air.

Any removal of lactic acid during recovery will result in the adjustment of the acid-base equilibrium in the tissues with a corresponding retention of carbon dioxide. In experiments which have been made on the respiratory exchanges during the recovery period [Slater, 1927] there appears a marked drop in the respiratory quotient; the carbon dioxide evolved being lower than under normal conditions in spite of the increased oxygen intake. If, however, the observed carbon dioxide retention is compared with that calculated from the oxygen debt—assuming the relationship between lactic acid burnt and lactic acid removed which has been obtained for vertebrate muscle, viz. 1 : 4.4 [Furasawa and Hartree, 1926]—it is found in most cases to amount to less than half.

The present communication deals with the determination of the lactic acid content of the insects before and after anaerobiosis, in an attempt to establish directly the relationship between lactic acid and oxygen debt, which has been foreshadowed by the study of the respiratory exchanges.

Duration of life under anaerobic conditions.

In the earlier experiments considerable variations were observed owing to small traces of oxygen in the nitrogen, which was then in use as the inert gas. It was subsequently found that the insects can utilise oxygen at very low partial pressures; thus nitrogen containing 1 % of oxygen was not sufficient to produce the condition resembling anaesthesia, which characterises the beginning of the anaerobic state.

More recent experiments carried out with carefully purified hydrogen yielded better results; at 18° the insects suffered permanent damage if they were deprived of oxygen for more than two hours, the same condition being reached at 25° in one hour. It will be seen from the subsequent determinations that the concentration of lactic acid is approximately the same in each case. The average length of life without air is therefore shorter than was at first believed, but individual cockroaches differ widely; after four hours at 18° it is sometimes possible to obtain complete recovery, although this is comparatively rare.

The only obvious symptoms arising from prolonged oxygen lack are a swelling of the gut due to the accumulation of gases, and an increase in the brittleness of the insects, the legs coming away from the body at the slightest touch.

Method used for the estimation of lactic acid.

The estimation of lactic acid in small animals protected by a heavy chitinous cuticle presents special difficulties. Such animals have a very small weight relative to their body size, and only by selecting at least ten specimens can a sufficient weight of tissue be obtained. Though the use of a number of insects for each experiment reduces the error due to individual variations, it greatly increases the difficulties connected with crushing and extraction. The extraction is further complicated by the difficulty of breaking up the cuticle in order to allow the extracting fluid to reach the tissues.

The cockroaches were caught in the building and used as soon as possible, in order to avoid errors due to any metabolic changes resulting from captivity. The selected insects were weighed and introduced rapidly into a short length of glass tubing about 2.5 cm. in diameter, closed at each end with a tightly fitting stopper. The tube was immersed in a thermostat at the required temperature, and a very rapid stream of hydrogen passed. The hydrogen was prepared in a Kipp's apparatus, washed by solutions of potassium hydroxide, potassium permanganate and silver nitrate, and finally passed through a glass spiral in the thermostat in order to raise the temperature to that required for the experiment.

If the stream of hydrogen was sufficiently rapid, and the wash bottles had been previously filled with hydrogen, only a slight struggle took place, the cockroaches becoming motionless within two minutes. After five minutes the stream of gas was slowed down to 15 to 20 bubbles per minute and maintained at that rate throughout the experiment.

In the preliminary experiments wide variations in the lactic acid content were obtained; these variations were far greater after the periods of oxygen lack than in the control experiments. Wigglesworth [1927] has shown that the gut of the cockroach develops considerable acidity due to the presence of yeasts and bacteria, and that the chief product is lactic acid. In the later experiments, therefore, the gut was removed before the lactic acid was estimated. The general results after removal of the gut were much lower, and the variations after anaerobiosis fell within the limits of error to be expected in this estimation.

The insects to be examined were broken up in a 4 % solution of trichloro-acetic acid, and the mash was allowed to stand. The clear filtrate from this was freed from sugar by the copper-lime method, and the lactic acid in the sugar-free solution estimated by Meyerhof's modification of the Clausen method. Duplicate determinations were made in each case, and the results rejected unless they were in reasonable agreement.

It was necessary before proceeding to the actual experimental work to ascertain the extent to which the tissue was being extracted. A number of estimations were made on whole cockroaches, using different methods of disintegration, the results of which are given below. It will be seen that mincing with a vegetable mincer is by far the most efficient method. This method was adopted throughout the later experiments.

Table I.

Method	Number of determinations	Mean of lactic acid found mg. %	% mean deviation
Grinding alone	3	8.5	5.9
Grinding with sand	6	28.8	10.4
Mincing with a vegetable mincer	5	40.9	7.1

There remained now only the length of time of extraction as a further possible source of error. Experiments were made in which the mash was allowed to stand for various times, and it was found that after 1 hour no further extraction occurred. This time was therefore fixed as the minimum time for extraction.

The cockroaches were selected as far as possible to weigh between 0.3 and 0.4 g. Sex was found to have no marked influence on the lactic acid content; it was therefore unnecessary to take any special precautions in this connection. In actual practice it was found convenient to use a preponderating number of females.

EXPERIMENTAL RESULTS.

Table II.

Series and temp.	Duration of anaerobiosis	Number of results	Lactic acid found mg. %	% mean deviation	Excess lactic acid		Ratio found calc.
					Found mg. %	Calc. mg. %	
I	Control	4	18.8	20	—	—	—
II 14°	1 hour	2	37.9	6	19.1	93.0	0.21
III 14°	2 hours	4	55.0	1.7	36.2	186.0	0.20
IV 18°	10 minutes	2	27.5	5.4	8.7	23.0	0.37
V 18°	1 hour	5	57.2	7.4	38.4	138.0	0.28
VI 18°	2 hours	3	80.6	2.3	67.8	276.0	0.25
VII 25°	1 hour	3	72.6	4.6	53.8	270.0	0.20
VIII 25°	2 hours	3	98.8	10.0	80.0	540.0	0.15

In Table II are summarised the results of the lactic acid determinations made immediately after a period of anaerobiosis. Experiments were made at three different temperatures, viz. 14°, 18°, and 25°. It was necessary to obtain a basal value for the lactic acid content of the cockroach after removal of the gut. In order to do so the cockroaches were lightly chloroformed and the guts

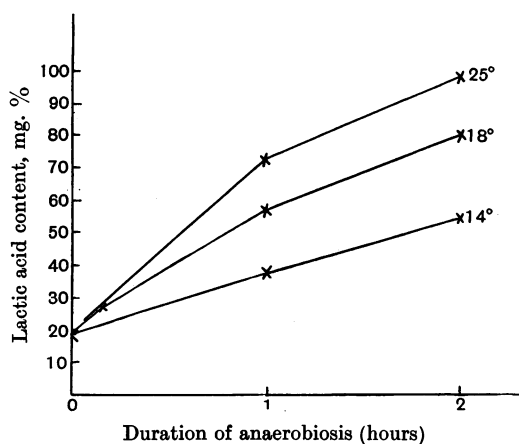


Fig. 1.

removed, the rest of the estimation following the normal method. The results of these determinations are given in the first series. Owing to the smallness of the value the mean percentage deviation is high. The figures given in Series IV were obtained in order to show that the slight struggle consequent on the admission of the hydrogen is responsible for no great production of lactic acid. It is difficult to control experiments over so short a period, and it would be unwise to place any absolute reliance on these figures, but they indicate quite clearly that the onset of anaerobiosis is accompanied by little or no lactic acid formation.

The remaining experimental figures require no special explanation. They show quite clearly that the concentration of lactic acid increases with the period of anaerobiosis, and also with the temperature at which the experiments are

carried out. Fig. 1 shows these relations graphically. The relation between the concentration of lactic acid and the period of anaerobiosis is for the lower values of lactic acid approximately linear. This relationship does not appear to hold for the higher values, there being a noticeable drop in output during the second hour at 25°, corresponding to the approach of the limit of anaerobic life.

It was now necessary to show that the lactic acid disappeared during recovery. As the result of experiments described in a previous communication [Davis and Slater, 1926] it was found that after 1 hour of anaerobic life at 25° a period of 3 hours was required before the whole of the oxygen debt had been recovered. A batch of cockroaches was exposed to hydrogen for 1 hour at 25°, and analyses made of a number of insects immediately after the admission of air, and at intervals of 1, 2 and 3 hours. The estimations were carried out in duplicate and the results are shown in Fig. 2, together with the graph of the oxygen debt recovery for purposes of comparison. It will be seen that the curves correspond within the limits of experimental error, the animals having returned to normal at the end of 3 hours.

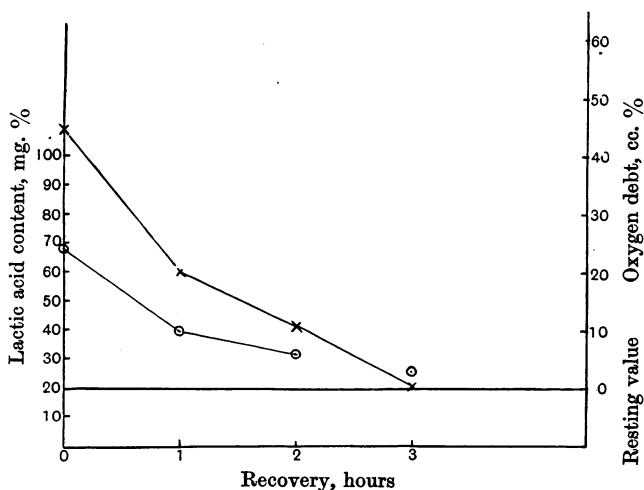


Fig. 2. × Oxygen debt ○ Lactic acid content

DISCUSSION.

The formation of lactic acid during anaerobiosis, and its removal during recovery, is in accordance with the original assumption that the energy required for the maintenance of life in the absence of oxygen is provided by the breakdown of glycogen to lactic acid. It is possible to check this hypothesis further by comparing the quantity of lactic acid found with that calculated from the respiratory exchanges.

If we assume that the body tissues of the cockroach behave in the same way as vertebrate tissues, so that for each molecule of lactic acid, or its carbohydrate equivalent, burnt, 4.4 molecules are removed, we can calculate the

amount of lactic acid which should be associated with a given oxygen debt. Further, the oxygen debt is known to be equal to the oxygen which would have been used during the period of anaerobiosis if the animals had remained at rest in air. The oxygen intake at rest for 25° has been measured, and for cockroaches of the type used the average figure is 450 mm.³ per g. per hour. Vernon [1897] has measured the carbon dioxide output at lower temperatures, and from his figures it is possible, taking the respiratory quotient of the freshly caught cockroach at 0.9, to calculate the oxygen debt for 14° and 18°.

The lactic acid values calculated in this way are given in Table II, together with the ratio lactic acid found/lactic acid calculated. It is quite clear from these figures that the assumptions do not hold quantitatively, yet the uniformity of the ratio suggests that the general thesis is in part correct.

The low lactic acid content of the cockroaches may be explained by assuming the oxidation of a larger proportion of the lactic acid; in fact the figures suggest its total oxidation with no accompanying recovery. In Table III are shown the values for lactic acid found compared with those calculated for complete oxidation.

Table III.

Series	Excess lactic acid found mg. %	Excess lactic acid calc. for complete oxidation mg. %	Ratio found calc.
II	19.1	20.7	0.92
III	36.2	42.2	0.86
IV	8.7	5.2	1.67
V	38.4	31.3	1.23
VI	67.8	62.5	1.08
VII	53.8	61.6	0.88
VIII	80.0	122.0	0.66

Av. 1.04

Except in abnormal cases of Series IV and VIII, the ratio of the lactic acid found to that calculated lies near to unity.

Support is given to this apparent absence of any recovery process by the abnormally high carbon dioxide output of the cockroach when compared with other animals. Thus, according to Vernon [1897], it is five times as great as that of the frog, and eight times as great as that of the earthworm, at 20°.

It is possible to suggest other explanations, but they do not fit so closely to the observed facts as the assumption that the whole of the lactic acid is burnt, without any accompanying recovery. For example, an extension and adaptation of the suggestion put forward by Gerrard [1927] in the case of isolated nerve, that an oxygen carrier is present in the tissue, would partly explain the facts, but it would not account for the linear relationship between lactic acid production and the period of anaerobiosis, or for the close agreement in time between the oxygen debt recovery and the disappearance of lactic acid. Moreover, the carrier would have to be present in quantities sufficient to deal with 240 mg. % of lactic acid.

Again, a second process at present unknown may occur simultaneously with the lactic acid formation. Without a complete analysis of the cockroaches it is not possible to set aside this explanation, although it is difficult to reconcile it with the close relationship between lactic acid production and oxygen debt.

Until further evidence is available it seems reasonable therefore to accept the complete oxidation of the lactic acid as a working hypothesis.

SUMMARY.

(1) The lactic acid content of cockroaches (*Periplaneta orientalis*) has been determined after various periods of anaerobiosis and during the recovery process.

(2) Lactic acid accumulates during anaerobiosis and returns to the resting value slowly during the recovery process.

(3) The excess lactic acid amounts to only about one-fifth of that calculated from the oxygen debt on the assumption that the recovery takes place as in vertebrate muscle.

(4) If, on the other hand, it is assumed that all the lactic acid is burnt without recovery, then the excess lactic acid agrees within the limits of experimental error with that calculated from the oxygen debt.

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REFERENCES.

- Davis and Slater (1926). *Biochem. J.* **20**, 1167.
Furasawa and Hartree (1926). *J. Physiol.* **62**, 203.
Gerrard (1927). *J. Physiol.* **63**, 280.
Slater (1927). *Biochem. J.* **21**, 198.
Vernon (1897). *J. Physiol.* **21**, 443.
Wigglesworth (1927). *Biochem. J.* **21**, 791.